Supporting information
CRISPR/Cas13a-Induced Self-Priming Cyclic Amplification Enables Liquid Biopsy of Exosomal Circular RNA in Non-small Cell Lung Cancer

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Supplemented experimental section

1. crRNA synthesis

The double-stranded DNA (dsDNA) template used for crRNA transcription was prepared by mixing equimolar amounts of T7 promoter-containing DNA template and its complementary strand. The mixture was denatured at 95 °C for 3 min and then annealed at 50 °C for 5 min to form the complete dsDNA structure. This resulting dsDNA served as the template for in vitro transcription of crRNA, which was performed using the T7 In Vitro Transcription Kit (Shanghai Yeasen Biotechnology) according to the manufacturer's protocol. The transcribed crRNA was purified with the RNAclean Kit (Tiangen Biotech) and quantified using a NanoDrop One spectrophotometer.

2. Cell culture, exosomes isolation, and exosomes characterization

A549 cells were cultured in RPMI-1640 medium with 10% fetal bovine serum (FBS) and maintained in a humidified atmosphere of 5% CO_2 at 37 °C. For exosome isolation, we washed the cells three times with phosphate-buffered saline (PBS) when cells reached 70% confluence and maintained them for an additional 12 h in medium without FBS; the culture medium was then collected for exosome isolation by

ultracentrifugation according to standard differential centrifugation separation protocols.

3. Total RNA extraction from A549 cells

A549 cells were maintained in DMEM medium supplemented with 10% (v/v) fetal bovine serum, 100 μg/mL streptomycin, and 100 U/mL penicillin, and cultured at 37 °C in a humidified incubator with 5% CO_2. Total RNA was extracted from the cells using TRIzol reagent (Invitrogen, Thermo Fisher Scientific) following the recommended procedure, and RNA concentration was determined with a NanoDrop One instrument.

4. Clinical samples treatment and target circRNA detection.

Exosomes were isolated from clinical blood samples to extract RNA and analyze the expression of hsa_circ_0003026. First, 5 mL of each sample (10 from NSCLC patients and 10 from healthy controls) was centrifuged at 5000g for 10 min and passed through a 0.22-μm filter to obtain cell-free plasma. Exosomes were then extracted using a commercial exosome isolation kit. After removing residual cells and debris by centrifugation, the samples were incubated overnight with an exosome precipitation solution. The resulting precipitate was re-suspended in exosome resuspension buffer for downstream processing. Total RNA was subsequently extracted and purified from the exosomes using an RNA extraction kit. The expression level of hsa_circ_0003026 was detected using both the proposed method and a conventional PCR-based approach. The relative expression was calculated by normalizing the results to the first healthy control sample, which was set as the reference.

Table S1. The sequences of the oligonucleotides used in this work.

Name	**Sequences (5'-3' direction)**
L-1	rGrGrCrUrCrArArUrArUrCrCrArUrGrUrCrUrUrCrCrArArCrGrUrC rUrCrCrArGrUrGrUrGrCrUrGrArUrCrUrUrCrUrGrArCrArUrUrCr ArGrGrUrCrUrUrCrCrArGrUrGrUrCrUrGrCrArArUrArUrCrCrArG
L-2	rGrGrUrUrUrCrCrGrArUrGrGrCrArCrCrUrGrUrGrUrCrArArGrGr UrCrUrUrCrCrArArCrArArCrUrCrCrGrGrGrUrCrUrUrCrC
H1	CGACAGCAGAGGATTTGTTGTGTGGAAGTGTGAGCGGATT TTCCTCTGCTGTCGTTTTTCCATCTCATCCCTGCGTGTCrUrUr UrUrU-C3 spacer
H2	ATCGTCGTGACTGTTTGTAATAGGACAGAGCCCCGCACTTT CAGTCACGACGATTTTTTGACACGCAGGGATGAGATGGrUr UrUrUrU-C3 spacer
crRNA	GAUUUAGACUACCCCAAAAACGAAGGGGACUAAAACCCG CCCAUUUGGCAGAAACAGCA
circRNA	CUCACACUUAACUCUGCUGUUUCUG||CCAAAUGGGCGGAC UGUUGAAGUGG
M1	CUCACACUUAACUCUGCUGUUUCUG||ACAAAUGGGCGGAC

	UGUUGAAGUGG
M2	CUCACACUUAACUCUGCUGUUUAUG||ACAGAUGGGCGGAC UGUUGAAGUGG
M3	CUCACACUUAACUCUGCUGCUUAUG||ACAGAUAGGCGGAC UGUUGAAGUGG

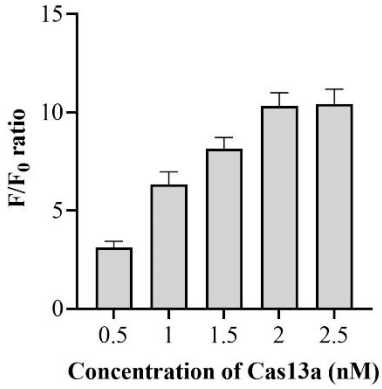

Fig S1. F/F_0 ratio of the method when different Cas13a concentations were used.

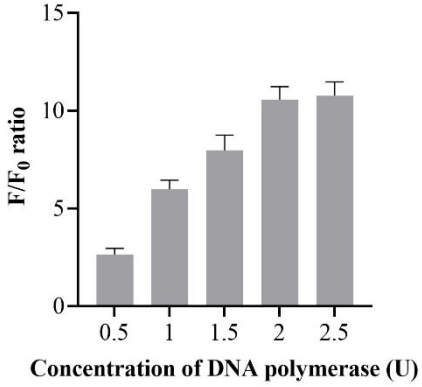

Fig S2. F/F_0 ratio of the method when different DNA polymerase concentrations were used.

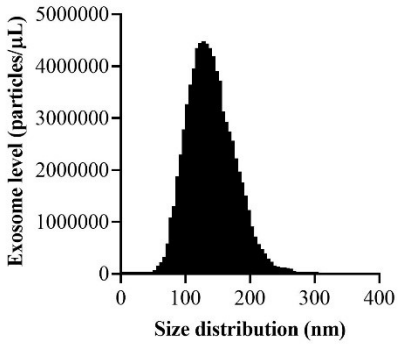

Figure S3. NTA result of the extracted exosomes.

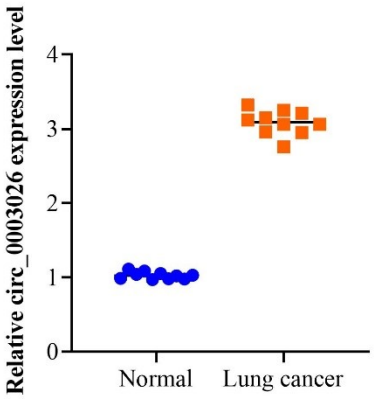

Figure S4. Relative target circRNA expression from normal and lung cancer samples by the PCR method.